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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
Recently, in the HMT-3522 cell line based model for breast tumor progression, TACC2 mRNA was shown to be downregulated in the more malignant clones of the series. This indicates that TACC2 downregulation is an important step in breast tumor progression. We have now shown that increased expression of TACC2 alters the *in vitro* cellular dynamics of breast cancer cell lines in a cell type specific manner. While TACC2 expression does not appear to inhibit cellular division, TACC2 does affect anchorage independent growth and cell migration. We have demonstrated that TACC2 interacts with hGCN5, a key component of transcriptional regulatory complexes. This suggests that TACC2 could play a role in the regulation of transcription through interaction with this molecule. The observation of specific effects of TACC2 expression in estrogen receptor negative compared to estrogen receptor positive cell lines may also support this idea. Therefore, further characterization of the role of the interaction of TACC2 with hGCN5 in transcription will elucidate how alterations in this complex could promote the malignant phenotype.

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Table of Contents

INTRODUCTION

The human transforming acidic coiled-coil (TACC) family of genes map to chromosomal regions associated with the development and progression of cancer (1, 2). TACC2 is normally expressed at low levels in normal breast cells (3). Recently, Chen et al demonstrated that TACC2 mRNA was downregulated in the more malignant clones of the HMT-3522 cell line based model for breast tumor progression (3). These authors also reported the cloning of a 3.8kb. TACC2 cDNA (named AZU-1), encoding a 571 amino acids protein, of predicted molecular mass 64kDa. Reintroduction of this cDNA into the malignant breast tumor clone reduced the ability of these malignant cells to grow and metastasize (3). Thus, this suggested that TACC2 is a breast tumor suppressor gene, downregulation of which may be an important step during breast tumorigenesis. To determine a functional role for TACC2, we used yeast two hybrid analysis to identify potential TACC2 interacting proteins. We identified a cDNA clone, which corresponded to the carboxyl terminus of the histone acetyltransferase, hGCN5, a key component of a complex which regulates transcription by acetylating histones and transcription factors (4, 5). This suggested that TACC2 could play a role in the regulation of transcription through interaction with this molecule. In the proposed studies, we will further characterize the normal functional role of TACC2, with particular relevance to its interaction with the histone acetyltransferases. This analysis will provide insights into the role of TACC2 in the normal growth and differentiation of cells, and possible mechanisms by which inactivation could promote tumor development.

This is the first year report for this grant covering the twelve month period from July 1/2001 –Jun 30/2002. In December 2000 the PI relocated his laboratory from the Cleveland Clinic to Roswell Park Cancer Institute. Unfortunately, Dr. Scott Howell remained in Cleveland, and his replacement, Dr Omkaram Gangisetty was only assigned in November 2001.

BODY

We have isolated the two major isoforms of TACC2 expressed during development, and identified the splice variants expressed in the mammary gland. We have now demonstrated using the available genomic sequence data that both AZU-1 and another TACC2 cDNA, ECTACC (6) contain cloning artifacts, thereby explaining the discrepancies in their sequences. In light of these findings, we have undertaken a reevaluation of the potential role of TACC2 as a breast tumor suppressor gene.

Specific aim 1. Analysis of the effect of full length TACC2 and deletion mutants on growth suppression of breast cancer cell lines.

To assess the potential growth suppressive role of the short TACC2 isoform (the major form expressed in the mammary gland) in breast cancer, we repeated the experiments of Chen et al (3) in two different human breast cancer cell lines. To investigate the consequences of increased expression levels of TACC2, we introduced a plasmid construct (EGTACC2), which expresses the short TACC2 open reading frame fused to the green fluorescent protein (EGFP) into MDA-MB-468 and MCF7. The construct was transfected into each cell line, and stable cell lines selected as previously described (1). Expression of the fusion protein was verified by fluorescence (Fig. 1), and western blot analysis demonstrated equivalent expression of the EGFP fusion protein (data not shown). TACC2 overexpression did not adversely affect the ability of

transfected MDA-MB-468 or MCF7 to proliferate, suggesting that overexpression of the TACC2 protein is not in itself toxic to the cells, or inhibitory to cell proliferation.

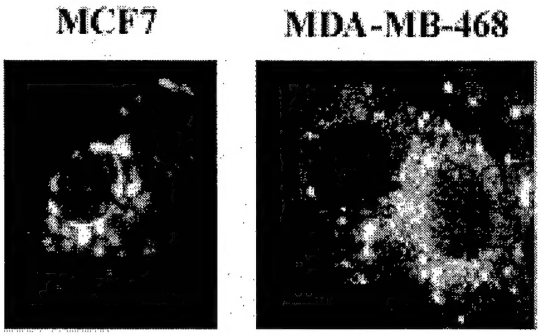


Figure 1: Expression of EGTACC2 transfected into MCF7 and MDA-MB-468.

An important indicator of whether a gene product is a potential oncogene or tumor suppressor is the ability of the gene to impart, or abolish anchorage independent growth *in vitro*. We were, thus, particularly interested in determining whether transfection of EGTACC2 into MCF7 and MDA-MB-468 cells would alter cellular motility and growth in soft agar. Interestingly, TACC2 overexpression produced no significant alteration in the ability of MCF7 to form colonies in soft agar (P=0.11) (Fig. 2). However, in the case of MDA-MB-468, the number of TACC2 overexpressing colonies was significantly reduced (P=0.01) when compared to controls (Fig. 2). MCF7 has previously been shown to migrate poorly through a basement membrane matrix (Matrigel), and transfection of EGTACC2 into MCF7 failed to increase the efficiency of migration. However, EGTACC2/MDA-MB-468 transfectants were significantly impaired in their ability to invade and migrate through the Matrigel matrix (P=0.001) (Fig. 3).

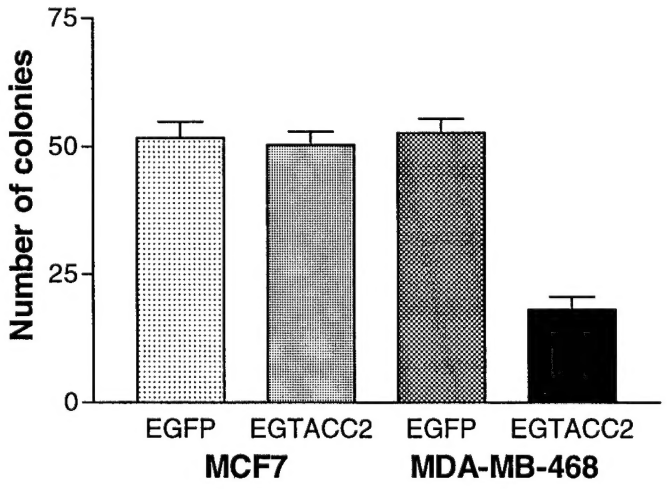
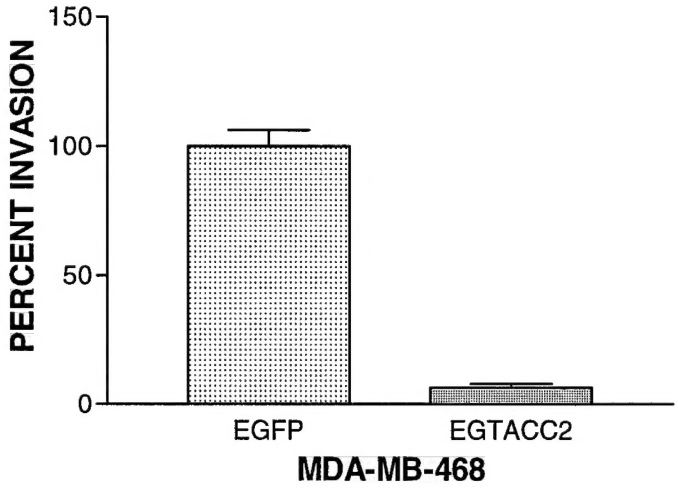


Fig. 2: Effect of overexpression of TACC2 on anchorage independent growth

Fig. 3. Effect of TACC2 on the invasive properties of MDA-MB-468



The reviewers of this grant suggested that we should examine potential changes in the expression of markers that are associated with breast cancer cells, or are characteristic of the differentiation of breast tumors. Based upon these suggestions, we next performed Western blot analysis to determine whether these phenotypic changes could be explained by downregulation of the matrix metalloproteinase MMP-9 and/or an increase in the expression of E-cadherin, and ZO-1. Increased expression of TACC2, however, failed to alter the expression of these proteins. Thus, although TACC2 may act to inhibit the invasion of some breast tumor cells through the basement membrane, this effect is not mediated by changes in expression of MMP9, or upregulation of components of tight junctions.

It has been proposed that TACC2 is a classII tumor suppressor, in that changes in expression, as opposed to mutations can be linked to breast tumor progression (3). Although we have not detected significant changes in TACC2 protein levels in breast cancer cell lines, or RNA levels in resected tumor samples, analysis of TACC2 overexpressing cell lines revealed cell type specific effects on the ability of breast cancer cells to exhibit anchorage independent growth and to migrate through a basement membrane like matrix. TACC2 did not alter the proliferation rates of MCF7 and MDA-MB-468 breast tumor cell lines in culture. TACC2 had little effect on the growth characteristics of the estrogen receptor positive MCF7 cell line, but significantly reduced the ability of estrogen receptor negative cell line MDA-MB-468 to grow in soft agar and to migrate through an extracellular matrix. This suggests that the effect of overexpression of TACC2 on the ability of breast cancer cells to divide in culture could be differentially affected by the genetic background of the original tumor. Interestingly, the HMT-3522 cell line model, in which a potential role of TACC2 in breast tumorigenesis was examined (3), also lack estrogen receptors (7). This suggests that TACC2 mediated decreases in malignant phenotype may be dependent upon the absence of the estrogen receptor, and thus may link the function of TACC2 to estrogen signaling.

The analysis of the effect of the short isoform of TACC2 in breast cancer cell lines completes Task 1, as outlined in the Statement of Work. Deletion constructs which contain only

the coiled coil, the SDP repeat (8) and a construct which is equivalent to the AZU-1 construct have been generated and are currently being transfected into MDA-MB-468 (Task 2).

Specific Aim 2: Examination of the role of hGCN5 and the TACC2-hGCN5 interaction in breast tumorigenesis.

To examine a potential role for hGCN5 in breast cancer, we have initially performed Western blot analysis of 10 breast cancer cell lines, using a commercially available hGCN5 antibody (Santa Cruz Biotechnology). We have detected relatively low levels of the 80kDa protein, in all lines tested. Interestingly, we have also shown that the closely related gene, pCAF is expressed in the same lines, and may therefore represent an alternative target for potential TACC2 mediated repression events. Further analysis of the role of hGCN5 will center upon the reintroduction of hGCN5 into breast cancer cells. We have cloned the hGCN5 open reading frame into pcDNA3, and are in the process of cloning hGCN5 into the EGFPC2 vector. Once the latter clone is constructed, we will repeat the experiments outlined above. The reviewers suggested that low transfection efficiency may hamper this analysis. We have also obtained a set of retroviral vectors, into which we can clone hGCN5, should we experience problems.

Task 3 initially included expression analysis, phosphorylation status and HAT activity of native hGCN5. However, the low levels of expression raise the possibilty that hGCN5 gene may be negatively regulated or even mutated in breast cancer. We believe that task 3 is largely completed by this observation.

SUMMARY OF STATUS OF TASKS OUTLINED IN THE STATEMENT OF WORK

Task 1	Complete
Task 2	In Progress
Task 3	Complete
Task 4	In progress
Task 5	In progress
Task 6	Due to commence 07/03
Task 7	Due to commence 01/03
Task 8	Due to commence 01/03
Task 9	Due to commence 07/03

KEY RESEARCH ACCOMPLISHMENTS

1) TACC2 acts to suppress metastatic potential, but not cellular proliferation
2) The antimetastatic effect is confined to estrogen receptor negative breast tumors
3) Demonstration of low level expression of hGCN5 in breast cancer cells.

REPORTABLE OUTCOMES

1) Development of breast cancer cell lines expressing the short isoform of TACC2
2) Molecular cloning, genomic structure and expression of the putative breast tumor suppressor gene, TACC2. B. Lauffart, O. Gangisetty and I.H. Still. *In preparation*

CONCLUSIONS

The human transforming acidic coiled-coil (TACC) family of genes map to chromosomal regions associated with the progression of cancer. We have now cloned full length TACC2 cDNAs corresponding to the two major isoforms expressed during development. TACC2 is expressed as a 120kDa protein in the normal mammary gland. Increased expression of TACC2 alters the *in vitro* cellular dynamics of breast cancer cell lines in an apparently cell type specific manner. While TACC2 expression does not appear to inhibit cellular division, TACC2 does affect anchorage independent growth and cell migration. This implies that functional inactivation of TACC2 is important in the development of breast tumor metastases, which are the main cause of death in patients. We have demonstrated that TACC2 interacts with hGCN5, a key component of transcriptional regulatory complexes. This suggests that TACC2 could play a role in the regulation of transcription through interaction with histone acetyltransferases. The observation of specific effects of TACC2 expression in estrogen receptor negative compared to estrogen receptor positive cell lines may also support this idea. The finding that breast cancer cell lines do not express high levels of hGCN5 suggests that downregulation or functional inactivation of hGCN5 may also be important in the development of breast cancer. Attempts are underway to create stable, hGCN5 overexpressing cell lines in order to further characterize the role of the interaction of TACC2 with this molecule in transcription and how alterations in this complex promote the malignant phenotype.

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